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EFFECT OF FACTORS OF PROLONGED SPACE FLIGHT ON CONDITIONS OF TORTOISE SKELETON

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16. Abstract After 60-90 day space flight mild osteoporosis developed in the epiphyses and metaphyses of long tubular bones of tortoises, which was not attended with reduced mineral saturation of the preserved bone tissue microstructures. The diminished strength of the cancellous bone of the epiphyses in tortoises after space flight is due to the reduced total mass of the bone substance and, possibly, to changes in the properties of its structure. The strength of the compact substance did not change under the effect of weightlessness.

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EFFECT OF FACTORS OF PROLONGED SPACE FLIGHT ON CONDITION OF TORTOISE SKELETON

By G. P. Stupakov, A. I. Volozhin, V. A. Korzhen'yants, V. S. Yagodovskiy, A. N. Polyakov, V. V. Korolev, and V. A. Elivanov

A study of the changes of the skeleton during weightlessness is an urgent /9* task as a consequence of the possible reduction in strength characteristics of the bones, and consequently, the resistance to the effect of impact g-forces, for example, during landing of the reentry equipment of the spacecraft.

The goal of this work was to investigate the ultimate strength of the compact and spongy substance of tortoises who endured a spaceflight lasting 19, 22, 60 and 90 days, in comparison with the condition of the mineral component and the quantitative indices for porosity of the osseous structures.

TECHNIQUE

A study was made on 30 tortoises Festudo Horsfieldii Gray of both sexes weighing 180-545 g. In the spacecraft the animals (10) were placed in metal boxes, and during the flight they did not receive food (group "spaceflight"). Under ground conditions a "synchronous experiment" was conducted with maintenance of unfed animals (8) in analogous boxes. The control turtles (12) were kept in

*Numbers in margin indicate pagination in original foreign text

a vivarium (group "vivarium control") and received the ration standard for these animals. The animals were killed within 2 days after landing by the administration of hexenal (100 mg per 100 g of weight). The humaral and femoral bones after skeletonization were stored in cold in 0.5% neutral Formalin until the moment of testing for strength, then were placed in 10% Formalin. No later than one week after death a study was made of the strength of the whole bones by testing them for static bending or the sawed-off distal epiphyses--for compression. The tests were conducted on a standard ZM-40 machine with the use of reverse. In the tests for bending the bone was placed on supports, where the distance between them was 15 mm. The middle of this distance coincided with the axis of the bending knife and the middle of the long bone. The moment of inertia was determined with the assumption that the external shape of the bone diaphysis in cross section and the shape of the medullary canal represent a regular ellipse. The distal epiphyses were tested for compression by loading with the help of a cylindrically shaped penetrator with area of the cross section 1 mm. Each epiphysis was loaded in the medial and lateral region, then the average amount of ultimate strength was computed. The calculation error for the indices of the strength-measuring device in the machine did not exceed 1% of the measurable load, the rate of movement of the active head of the machine in the tests for bending was 35 mm/min, for compression 1 mm/min. Bones of animals were tested for bending after 22-and 60-day experiment, for compression after the 19-and 90-day. To compute the ultimate strength of the bones the external and internal dimensions of the bones were determined in their cross section from x-ray photographs taken in sagittal and frontal projection on the "Mikrat-200" film without an intensifying screen. Osteometry was conducted in the middle of the bone length with the help of an MBS-2 microscope equipped with an ocular-micrometer (measurement accuracy ± 0.025 mm).

The content of mineral component is a unit of osseous substance volume (mineral saturation in grams per 1 cm^3), correlation of mineral and organic components according to weight (ash content in %) and specific weight (density in grams per 1 cm^3) were determined in a fragment of the diaphysis cut out in the form of a ring, and in the distal epiphysis according to the technique previously described [3]. The degree of mineralization of the bone marrow diaphyses of the tortoise was studied after the 22-and 90-day flight by the method of quantitative micro-x-ray diffractometry of microsections [4]. In each micro-x-ray photograph the mineral saturation was evaluated at 10-20 points, and the average index for mineralization of the microstructures was computed.

For a histological study the osseous material was fixed in 10% neutral Formalin, decalcinated in 10% nitric acid. The celloidin media were stained with hematoxylin-eosin and pyrofuschin according to Van Gieson. The area of the vascular canals was measured on the decalcinated cross sections (thickness 7-10 μm) of the diaphyses after using a grid [1], installed in the eye piece (x7) of the microscope with lens x40. One point on the grid (conventional unit of area) /10 corresponded to 53 μm^2. On each peparation 50-100 canals were measured. All the vascular canals were arranged according to sizes: 5, 6-10, 11-15, 16-20, 21-40, 41-60, 61-100 con. un. The number of canals in each row was expressed in percents of the number of all those measured on the preparation. A separate calculation was made of the total number of medullary spaces (with area 100 con. un.), arranged primarily on the side of the endosteum.

RESULTS AND THEIR DISCUSSION

All the indices in the groups "synchronous experiment" and "vivarium control" did not differ, and therefore these animals were united into one control

group (20 tortoises). Since the weight of the animals fluctuated in broad limits, a correlation analysis was made of the weight indices of the control animals with the amounts of mineral saturation, ash content and density of the spongy and compact substance of the femoral and humeral bones. It was established, that there is no link between the indicated amounts and the weight of the animals. A close dependence was revealed of the mineral saturation on the ash content amount, which can indicate the relative constancy in the dimensions and the total volume of microscopic planes in the bones of different tortoises. A close correlation was also revealed between the ash content indices of the compact and spongy substance.

The link between ash content and mineral saturation and density of the spongy substance was pronounced considerably more weakly than in the compact. This is explained by the fact, that the volumetric content of the mineral component in the spongy substance depends not so much on the degree of mineralization of the osseous substance of the trabeculae (with its relative constancy), as on the density of their composition. High correlation coefficients for the indices of mineral saturation and density of the spongy and compact substance can be explained by the fact, that they both depend on the weight of the mineral component in a unit of volume of osseous tissue.

All the tortoises who experience the spaceflight were united into two groups: 1--six animals after the 19-22-day flight, second--four tortoises after the 60- and 90-day flight (Table 1).

The properties of the compact osseous tissue did not depend on the duration of weightlessness, no signs of osteoporosis were found by physical methods. In the distal epiphyses in the animals after the 19-22-day flights, the amounts /11

of mineral saturation, ash content and density also did not change. After the 60-90-day flights the density, and especially the mineral saturation had a tendency to decrease (see Table 1). No decrease in the amount of ash content was revealed.

TABLE 1. MINERAL SATURATION, ASH CONTENT AND DENSITY OF COMPACT AND SPONGY OSSEOUS TISSUE IN TORTOISES ($M \pm m$)

Examined	Diaphysis			Epiphysis		
	Mineral Saturation, g/cm³	Ash Content %	Density, g/cm³	Mineral Saturation, g/cm³	Ash Content %	Density g/cm³
	Femoral Bone					
Control	1.168±0.014	60.7±0,6	1.923±0.012	0.354±0.008	57.4±0.7	0.613±0.016
Space flight:						
19 and 22 days	1.192±0.005 (102.2)	61.5±0.4 (101.2)	1.958±0.010 (101.8)	0.346±0.014 (97.8)	59.1±0.3 (102.9)	0.591±0.021 (96.5)
60 and 90 days	1.175±0.008 (100.6)	61.1±1.5 (100.6)	1.865±0.013 (97.2)	0.326±0.017 (92.1)	56.1±0.4 (97.7)	0.580±0.025 (94.5)
	Humeral Bone					
Control	1.174±0.013	60.9±0.4	1.911±0.012	0.413±0.010	58.0±0.7	0.710±0.015
Space flight:						
19 and 22 days	1.163±0.013 (99.2)	60.7±0.6 (99.6)	1.929±0.013 (101 4)	0.418±0.014 (101.1)	59.8±0.3 (103.1)	0.708±0.024 (99.7)
60 and 90 days	1.150±0.016 (98.0)	61.2±0.7 (100.4)	1.881±0.017 (99.0)	0.387±0.014 (93.7)	57.5±0.3 (99.3)	0.686±0.021 (96.6)

Note: In parentheses the same indices are given in % of the control.

According to the data of a micro-x-ray diffractometry study, the average level of mineralization of the osseous tissue in the diaphyses of the bones after the 22-day spaceflight was close to the data of the control--1.33 and 1.36 mg/mm^3 respectively. After the 90-day flight no changes were also found in the mineralization of the microstructures of the osseous tissue (1.39 and 1.31 mg/mm^3).

Physical methods reveal the development of slight osseoperosis in the epiphyses and metaphyses of the femoral and humeral bones after 60-and 90-day

flights. In a histological study these data were confirmed. During morphometry of the vascular canals certain laws were revealed that govern the change in dimensions depending on the duration of the experiment. In the control tortoises asymmetrical distribution of the vascular canals over their area was established (Figure 1). 63% of all the canals of an area up to 10 con. un. The largest canals are localized on the side of the endosteum, where apparently, the most intensive processes of bone reconstruction occur with the dominance of resorption. The asymmetrical distribution of canals indicate the fairly intensive reconstruction [6] of the cortical layer of the long tubular bones of the tortoises. After the 19-22-day spaceflight no significant changes were noted in the distribution of vascular canals over the area. However, in the animals of this group a decrease up to 7.7 ± 1.4 (in the control 11.3 ± 0.9) was found in the number of spaces delimited by the trabeculae from the medullary canal. Apparently, this result is due to activation of the resorptive processes on the part of the endosteum. In the tortoises after 60-day flight a more than double reduction as compared to the control was noted in the relative number of smallest vascular canals (up to 5 con. un.) due to canals with area of 11-60 con. un. The relative reduction in the number of canals with area up to 5 con. un was observed also after the 90-day flight, when there was a considerable rise in the relative number of canals with size from 5 to 10 con. un., while the frequency of canal distribution with area 16-100 con. un. did not differ from that in the control. Under conditions of 60-90-day experiments the total number of medullary spaces in the wall of the diaphysis was normalized (respectively 10.0 ± 1.4 and 11.5 ± 0.3). This fact, probably is explained by the emergence of foci of resorption of the cortical layer of the diaphysis. One can draw the conclusion, that a 20-day spaceflight induces intensification of resorption of only the

"most accessible" trabeculae, on the side of the medullary canal. During a 60-day flight the sizes of the "average" (11-60 con. un.) canals are increased; the majority of them are located in the intermedial zone of the cortical layer, while after the 90-day experiment the resorptive process also affects the zone of the diaphysis wall near the external surface of the bone, where the majority /12 of canals are located with small area (up to 5 con. un.). The constantly continuing resorption of osseous trabeculae on the part of the medullary canal results in a seeming normalization in the number of medullary spaces within the diaphysis wall limits.

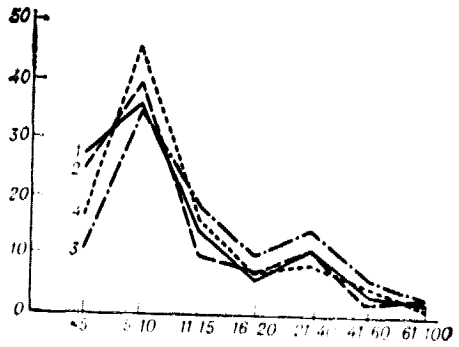

Figure 1. Frequency of Distribution of Vascular Canals Over Area in Diaphyses of Humeral and Femoral Bones of Tortoises

Key:
1. control
2,3,4. spaceflight respectively 19-22, 60 and 90 days

On x axis--area of canals in con. un., on y axis--percentage of canals of given area of total number of canals

A correlation analysis did not establish any significant link between the indices of mineral saturation, ash content and density of the compact substance with its ultimate strength in the control animals (Table 2). This result is due not to the absence of such a link, but apparently, the insignificant individual fluctuations in the indices for the osseous mineral phase and its ultimate strength. In the control animals, the ultimate strength of the bone was 15.24 ± 0.61 kg-f/mm^2, in the 22-and 60-day flight animals--respectively 14.18 ± 0.53 and 15.19 ± 0.81 kg-f/mm^2. Consequently, the strength of the compact substance in the diaphysis of the femoral and humeral bones in tortoises after 22-and 60-day flights was not changed, which agrees with an absence of a change

in the condition of the mineral component in the indicated sections of the long bones. A correlation analysis (see Table 2) in the control tortoises revealed a very close link between the ultimate strength of the spongy substance in the distal epiphysis of the tubular bone and the amounts of mineral saturation and density in the absence of a dependence on ash content. Consequently, the strength of the spongy structure is mainly determined by the quantity of osseous substance in a unit of bone volume and to a small degree, the correlation of mineral and organic components. Since the dependence of ultimate strength on mineral saturation is distinct and bears an exponential nature (Figure 2), a comparison of the average strength indices in groups with a small number of /13 observations is impermissible, and a comparison with the control (12 tortoises) of the amounts of strength in two tortoises after 90-day or 4 tortoises after the 19-day flight is unsubstantiated. A unification of the indices of both experimental groups into one (6 animals) makes such a comparison more substantiated, although such a grouping is not quite successful according to the experimental periods. Nevertheless, the ultimate strength of the spongy substance in tortoises after the 19-90-day flight on the average equalled 3.28 ± 0.34 kg-f/mm^2, and was 20% lower than in the control animal--4.11 ± 0.45 kg-f/mm^2.

TABLE 2. CORRELATION COEFFICIENTS FOR INDICES OF MINERAL SATURATION, ASH CONTENT AND DENSITY OF SPONGY AND COMPACT OSSEOUS TISSUE OF FEMORAL AND HUMERAL BONE IN CONTROL TORTOISES WITH AMOUNTS OF ULTIMATE STRENGTH OF OSSEOUS TISSUE

	Osseous Tissue of Diaphysis			Osseous Tissue of Epiphysis		
	Mineral Saturation g/cm^3	Ash Content %	Density, g/cm^3	Mineral Saturation g/cm^3	Ash Content %	Density, g/cm^3
Ultimate strength of bone, kg-f/mm^2:						
osseous tissue of diaphysis	0.30	0.59**	0.20	-	-	-
osseous tissue of epiphysis	-	-	-	0.86*	0.21	0.92*

*P<0.001; **P<0.01

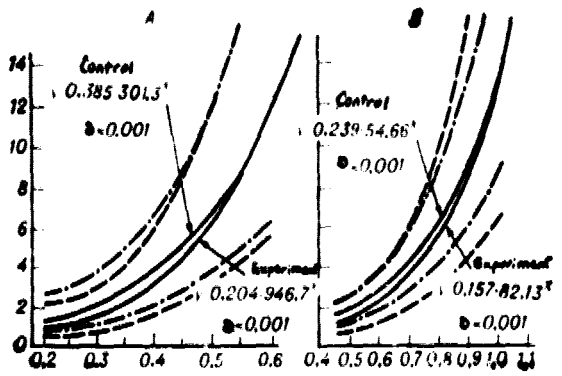

Figure 2. Change in Dependence of Ultimate Strength of Spongy Substance in Epiphyses of Femoral and Humeral Bones on its Mineral Saturation and Density in Tortoises who Endured 19-and 90-Day Spaceflights

Boundaries of 95% confidence interval are designated in the experimental group by dotted line, for the control by a dash-dot.

On the x axis: A--mineral saturation, B--density (in g/cm^3);

On the y axis--ultimate strength

It was revealed, that with the same amount of mineral saturation in the tortoise bones of both groups the strength of the bone is lower in the experimental than in the control animals. Regressions of "mineral saturation-strength" in the control ($lgy=0.424+2.501 \cdot x$) and experimental ($lgy=-0.689+2.976 \cdot x$) groups are significantly different, which was confirmed by verification of the hypothesis on the values of the constants ($t_a=21.12$, $P<0.001$) in the absence of differences in the residual dispersion [5]. In a comparison of the regressions "density-strength" the residual dispersions in both groups (experimental-$lgy=-0.621+1.738 \cdot x$; control -$lgy=-0.805+1.94 \cdot x$) significantly differed in both groups $F=4.06$, $P<0.01$. For this case there is yet no precise method for further comparison of the regressions [5].

The obtained relationships in the tortoises who underwent a spaceflight can indicate the decrease in the strength characteristics of the structure of spongy bone tissue not only due to a decrease in its weight in a unit of volume, i.e., as a consequence of the development of osteoperosis, but also as a result of some additional changes in the substance of the bone during its reconstruction

in the new functional conditions of weight unloading of the skeleton. Such a viewpoint is confirmed by the fact, that with an increase in the mineral saturation and density of the osseous substance, and consequently, with a decrease in the rate of physiological construction of the bone the relationships "mineral saturation-strength" and "density-strength" emerge onto the control level.

The results of the work make it possible to draw certain generalizations. First, under the influence of spaceflight factors lasting 60-90 days, in tortoises in the epiphyses and metaphyses of the long tubular bones slight osteoporosis develops. Second, the resorption of the osseous substance is not accompanied by a decrease in the mineral saturation of the preserved microstructures, which can explain the data on the absence of changes in the microhardness of the osseous tissue of cosmonauts [2]. A decrease in the strength of the spongy bone of the epiphyses in the tortoises after a spaceflight is linked to the decrease in the total weight of the osseous substance, and possibly, to a change in the properties of its structure. The strength of the compact substance is not altered. The development under conditions of weightlessness of osteoporosis and decrease in bone strength in tortoises indicates that the problem of the effect of spaceflight factors on the skeleton is extremely urgent.

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